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Short communication

Serological survey of *Ehrlichia* and *Anaplasma* infection of feral raccoons (*Procyon lotor*) in Kanagawa Prefecture, Japan

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Abstract

Numbers of feral raccoon; the possible reservoir animal of *Ehrlichia* and *Anaplasma*, are increasing in Japan. Thus serological methods were utilized to examine *Ehrlichia* and *Anaplasma* infection in raccoons from Kanagawa Prefecture, Japan. By using an indirect immunofluorescence assay, among 187 feral raccoons examined, 1 (0.5%) serologically reacted with *Ehrlichia canis*, 3 (1.6%) with *Ehrlichia chaffeensis* and 1 (0.5%) with *Anaplasma phagocytophilum* with the titers of 1:40 or more. Although screening PCR for *Ehrlichia* and *Anaplasma* species failed to detect the presence of ehrlichial DNA in serum samples, results of the serological tests suggested that the feral raccoons might be infected with some species of *Ehrlichia* and *Anaplasma*.

Keywords: *Anaplasma*; *Ehrlichia*; Raccoon

1. Introduction

The raccoon (*Procyon lotor* (Linnaeus, 1758)) is widely distributed throughout regions ranging from Canada to Central America. However, a large number of raccoons have been imported from the U.S.A. as pet animals into Japan since the 1970s. The intentional release and escape of pet raccoons has resulted in a naturalized population in most parts of Japan. One of the strongest concerns about the establishment of this animal in Japan is the possible transmission of pathogens to both human and domestic animals, because these animals were imported without sufficient quarantine until a new regulatory law was passed recently to control imported animals. Indeed, some emerging pathogens have been detected, including a *Babesia microti*-like parasite in Hokkaido (Kawabuchi et al., 2005) and gastrointestinal helminthes in Wakayama Prefecture (Sato and Suzuki, 2006).

Both *Ehrlichia* and *Anaplasma* are important tick-borne bacteria of both humans and animals. Especially *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* cause two major human infections, human monocytic ehrlichiosis and human granulocytic anaplasmosis, respectively (Anderson et al., 1991; Bakken et al., 1994). Because the feral raccoon is one of the reservoir animals of both *E. chaffeensis* and *A. phagocytophilum*

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in the U.S.A. (Comer et al., 2000; Levin et al., 2002), these ehrlichial pathogens might have been introduced into Japan by the imported animals. But little information is available on *Ehrlichia* and *Anaplasma* infection of feral raccoons in Japan. Thus the aim of this study was to examine the sero-prevalence of antibodies against *Ehrlichia* and *Anaplasma* in raccoons in Japan.

2. Materials and methods

2.1. Raccoon sera

From October 2001 to September 2002, a total of 187 raccoons (145 adults and 42 juveniles) were captured by cage traps in Kamakura, Fujisawa, Zushi, Sagamihara, Odawara and Shiroyama areas in Kanagawa Prefecture, Japan. Before sample collection, the general body condition of raccoons was examined thoroughly. Raccoons were immobilized by administering an intramuscular injection of ketamine hydrochloride and xylazine. After immobilization, the sex of the individual was noted and the animals were differentiated to two age groups such as adults and juveniles by the general appearance of the animals and the condition of the teeth. Blood samples were collected from the jugular or saphenous veins of raccoons. The blood samples were clotted for 1–2 h at room temperature and then centrifuged at 500 × *g* for 15 min. The separated sera were stored at −20 °C until analysis.

2.2. Indirect immunofluorescence assay (IFA)

A modified method of IFA was carried out to detect antibodies against *E. chaffeensis*, *Ehrlichia canis*, and *A. phagocytophilum*. IFA antigen slides were prepared using standard methods (Brouqui et al., 1994) using DH82 cells infected with *E. chaffeensis* (Arkansas strain, supplied by J. Dawson) and *E. canis* (Israel strain, supplied by Dr. Harrus, The Hebrew University of Jerusalem), and HL60 cells infected with *A. phagocytophila* (HGE agent Webster strain, supplied by J.S. Dumler). The raccoon sera samples were screened at a 1:20 dilution in phosphate-buffered saline (pH 7.2) Tween 0.5% (PBST). The antibody against the raccoon sera was prepared as follows: raccoon immunoglobulin was inoculated into a rabbit, and then the sera purified from the rabbit were used as second antibody. This anti-raccoon rabbit serum was kindly provided by the Department of Veterinary Science, National Institute of Infectious Diseases, Japan. Fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG conjugate (Cappel Co. Ltd., USA) was also used as the third antibody of the IFA. Reactive antibodies were then detected using a fluorescence light microscope. Those samples that reacted with any one of the antigens at the screening dilution were then titrated to endpoint. Because the positive and negative controls of raccoon sera were unavailable, serum from mice that were experimentally infected with *E. chaffeensis* and *A. phagocytophilum* were used as positive controls. Serum from a dog naturally infected with *E. canis* was also used as a positive control. FITC-labeled rabbit anti-mouse IgG conjugate and FITC-labeled rabbit anti-dog IgG conjugate were used as the second antibodies for the positive controls of *E. chaffeensis* and *A. phagocytophilum*, and *E. canis*, respectively.

When the serum showed a positive result with any one of the antigens at the dilution of 1:20, the reactivity of the serum with mouse spleen infected with *Ehrlichia muris* (Hyogo strain, supplied by Dr. Masayoshi Tsuji, Rakuno Gakuen University, Japan) and *Ehrlichia* from *Ixodes ovatus* (EIO) (HF639) were also examined by the method previously described (Watanabe et al., 2004).

2.3. PCR screening

When the serum showed a positive result with any one of the antigens at the dilution of 1:20, DNA was also extracted from the serum samples that showed any positive result, by using a QIAmp DNA Mini Kit (Qiagen, Hilden, Germany). Screening PCR for *Ehrlichia* and *Anaplasma* was performed by using the primer pair of EHR16SD and EHR16SR, which can amplify the 16S rRNA gene of genus *Ehrlichia*, *Anaplasma* and *Neorickettsia* (Parola et al., 2000).

3. Results and discussion

Among 187 feral raccoons examined, 9 (4.8%) were reactive to *E. chaffeensis*, *E. canis* or *A. phagocytophilum* at the screening level. All the positive samples were from adult individuals; the percent of positivity among 145 adult raccoons was 6.2%. The results of the titration of these nine samples are shown in Table 1. All the nine samples that reacted serologically with *E. chaffeensis*, *E. canis* or *A. phagocytophilum* were negative in the PCR to detect *Ehrlichia* or *Anaplasma*.

Of these nine samples, three (Nos. 13-18, 13-94, 13-180) showed the highest titers (1:40) with *E. chaffeensis*, but these samples also reacted with *E. canis*, *E. muris* or EIO at similar titers of 1:20. It has been previously demonstrated that cross-reactivity between the different *Ehrlichia* species may occur (Brouqui et al., 1992, 1994; Dumler et al., 1995). The